

for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. These techniques are useful for preparing "knock outs" having no functional GLUTX gene.

5 Once transgenic animals have been generated, the expression of the recombinant GLUTX gene can be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to determine whether integration of the transgene has taken

10 place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and RT-PCR. Samples of

15 GLUTX gene-expressing tissue can also be evaluated immunocytochemically using antibodies specific for the GLUTX transgene product.

For a review of techniques that can be used to generate and assess transgenic animals, those of ordinary skill in the art can consult Gordon (*Intl. Rev. Cytol.* 115:171-229, 1989), and may obtain additional guidance from, for example: Hogan *et al.* "Manipulating the Mouse Embryo" (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1986); Krimpenfort *et al.*, *Bio/Technology* 9:86, 1991; Palmiter 25 *et al.*, *Cell* 41:343, 1985; Kraemer *et al.*, "Genetic Manipulation of the Early Mammalian Embryo," Cold Spring Harbor Press, Cold Spring Harbor, NY, 1985; Hammer *et al.*, *Nature* 315:680, 1985; Purcel *et al.*, *Science* 244:1281, 1986; Wagner *et al.*, U.S. Patent No. 5,175,385; and Krimpenfort 30 *et al.*, U.S. Patent No. 5,175,384.

The transgenic animals of the invention can be used to determine the consequence of altering the expression of GLUTX in the context of various disease states. For

example, GLUTX knock out mice can be generated using an established line of mice that serve as a model for a disease in which activity of the missing gene is impaired.

5 **IV. Anti-GLUTX Antibodies**

GLUTX polypeptides (or immunogenic fragments or analogs thereof) can be used to raise antibodies useful in the invention; such polypeptides can be produced by recombinant techniques or synthesized (see, for example,

10 "Solid Phase Peptide Synthesis," *supra*; Ausubel *et al.*, *supra*). In general, GLUTX polypeptides can be coupled to a carrier protein, such as KLH, as described in Ausubel *et al.*, *supra*, mixed with an adjuvant, and injected into a host mammal. Antibodies produced in that animal can then be 15 purified by peptide antigen affinity chromatography.

In particular, various host animals can be immunized by injection with a GLUTX polypeptide or an antigenic fragment thereof. Commonly employed host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants

20 that can be used to increase the immunological response depend on the host species and include Freund's adjuvant (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, 25 keyhole limpet hemocyanin, and dinitrophenol. Potentially useful human adjuvants include BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules that are contained in the sera of the immunized animals.

30 Antibodies within the invention therefore include polyclonal antibodies and, in addition, monoclonal antibodies, humanized or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, and molecules

produced using a Fab expression library.

Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be prepared using the GLUTX polypeptides described above and 5 standard hybridoma technology (see, for example, Kohler *et al.*, *Nature* 256:495, 1975; Kohler *et al.*, *Eur. J. Immunol.* 6:511, 1976; Kohler *et al.*, *Eur. J. Immunol.* 6:292, 1976; Hammerling *et al.*, "Monoclonal Antibodies and T Cell Hybridomas," Elsevier, NY, 1981; Ausubel *et al.*, *supra*).

10 In particular, monoclonal antibodies can be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture such as described in Kohler *et al.*, *Nature* 256:495, 1975, and U.S. Patent No. 4,376,110; the human B-cell hybridoma 15 technique (Kosbor *et al.*, *Immunology Today* 4:72, 1983; Cole *et al.*, *Proc. Natl. Acad. Sci. USA* 80:2026, 1983), and the EBV-hybridoma technique (Cole *et al.*, "Monoclonal Antibodies and Cancer Therapy," Alan R. Liss, Inc., pp. 77-96, 1983). Such antibodies can be of any immunoglobulin class including 20 IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. The ability to produce high titers of mAbs *in vivo* makes this a particularly useful method of production.

25 Once produced, polyclonal or monoclonal antibodies are tested for specific GLUTX recognition by Western blot or immunoprecipitation analysis by standard methods, for example, as described in Ausubel *et al.*, *supra*. Antibodies that specifically recognize and bind to GLUTX are useful in 30 the invention. For example, such antibodies can be used in an immunoassay to monitor the level of GLUTX produced by a mammal (e.g., to determine the amount or subcellular location of GLUTX).